tion is absent have greatly reduced numbers of stomata, as in the dodders (Cuscuta) and the little mistletoes (Razoumofskya), while they are present in abundance in green parasites (Viscum and Phoradendron).

From the foregoing rapid and quite summary survey of the different phases of this question we are warranted in concluding:

1. That one of the functions of stomata is the admission of carbon dioxide to the chlorophyll-bearing tissues of the plant, for use in the formation of the carbohydrates.

2. That the loss of water by terrestrial plants was originally hurtful, and is so now in many cases.

3. That if plants have utilized this constant phenomenon it is for the supply of food matters of secondary importance, as the salts in solution in the water of the soil.

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*RECENT PROGRESS IN AGRICULTURAL CHEMISTRY.**

I.

SINCE the last *résumé* of progress in agricultural chemistry was reported to this body a considerable advance has been made in our knowledge of the methods and means of nitrogen assimilation. The most marked progress has been made along the line of the inoculation of seed and the soil with nitrifying ferments. Much has been done in this direction, and the results of the experiments are sufficiently encouraging to warrant the belief that much good may yet come to agriculture by following out this line of investigation. In 1895, in the Yearbook of the Department of Agriculture, the following statements occur:

"It may not be long until the farmer may apply to the laboratory for particular nitrifying ferments to be applied to such special purposes as are mentioned above. Because of the extreme minuteness of these organisms the too practical agronomist may laugh at the idea of producing fertility thereby, and this idea, indeed, would be of no value were it not for the wonderful facility of propagation which an organism of this kind has when exposed in a favorable environment. It is true that the pure cultures which the laboratory affords would be of little avail if limited to their own activity, and it is alone in the possibility of their almost illimitable development that their fertilizing effects may be secured."

It cannot be said that the prophecies foreshadowed in the above quotation have been fully verified, but at least something has been accomplished.

From the time that it was demonstrated by Hellriegel and Wilfarth that the power which leguminous plants possess of increasing their stores of nitrogen was due to the bacteria inhabiting nodules on their rootlets, the study of this phenomenon has been pushed with great vigor in all parts of the world. Intimately related, as it is, to the nitrifying organisms of the soil, it has, nevertheless, been demonstrated that the two species of bacteria, the general nitrifying species and the special so-called symbiotic species, inhabiting the roots of plants, are entirely different in their nature, and that their activity is not mutually convertible.

The most extensive experiments in the direct inoculation of the soil with nitrifying ferments have been conducted by Dr. Salfeld, of Lingen, in Hanover. The greater part of the experiments has been made on peaty soils, as it is in such soils that the greatest deficiency of nitrifying organisms is observed. An excellent review of Dr. Salfeld's work has been pub-

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lished by N. H. J. Miller, in the Journal of the Royal Agricultural Society of England, Vol. 7, third series, part 2, pp. 236 *et seq.*

The method employed by Dr. Salfeld was to spread upon the peaty soils large quantities of soils in which peas, beans and other leguminous crops had been grown. Immense quantities of the soil to be used to develop the fertilizing ferments were required; quantities ranging from 16 to 32 hundred weight per acre were used, and it was found that the larger quantities gave the better results.

The particular bacterium which is most active in developing nodules on plants has been called the *Bacillus radicicola.* It was found in Dr. Salfeld's experiments that there exist many peaty soils which are so poor in this bacterium as to require inoculation with other soils containing it before leguminous crops can be grown successfully.

As was to be expected, the most striking results were obtained with soils which were most deficient in the nitrifying bacillus, and when the inoculation was accompanied with the addition of a sufficient quantity of lime, phosphoric acid and potash. The large quantities of soil which are required for the direct inoculation, as outlined above, have rendered of great interest the attempts to secure inoculation in a more direct and positive manner. This has led to a study of the possibilities of securing pure cultures of nitrifying organisms which can be applied directly to the seed before planting, or can be mixed with moderate quantities of soil and thus distributed over a large area.

The most extensive experiments in the processes of seed inoculation have been carried on by Professor Nobbe, of Tharand, Saxony. The principle of these inoculations is first to secure the pure cultures of the bacteria inhabiting the nodules of the roots. These pure cultures are obtained by the ordinary bacteriological processes now so well known. With these pure cultures inoculations of various kinds have been practiced, viz., inoculations of the soil itself, inoculations of the exterior of the seed, and inoculation by pricking the seed with needles bearing the germs of the pure cultures.

The remarkable fact has been developed that while the bacteria derived from the pure cultures of the root nodules of various legumes appear to be microscopically identical they, nevertheless, have very distinct characters. The results of these experiments have shown that in inoculation best results are obtained when plants of the same species and, as nearly as possible, the same family are used. Even among the Leguminosæ, when passed from one species to another, the vitality of the organism is either diminished or entirely destroyed. This is illustrated, for instance, in attempts which have been made to inoculate the members of the pea family with the bacteria taken from the roots of clover, or *vice versa.* The commercial outcome of these experiments is that these cultures have been prepared on a large scale for general sale. While the idea of thus preparing fertilizers in a practically infinitesimal quantity for field work is not a new one, and is not lacking in its appeals to the imagination, it cannot be said that the practical results have been fully equal to the expectations which have been aroused.

The commercial name of these preparations is nitragin, although, etymologically, probably the term nitrogene would have been preferable, but it was necessary to distinguish it in some way from the name of the element.

The Imperial Seed Control Station at Vienna made experiments with 100 kilograms of soil taken from a field where lupines had been grown, and 20 kilograms of analogous soil coming from a field where

serradella had been grown. These soils, which were to be used for inoculation, were taken from portions of the field where the roots were abundantly provided with the usual nodules. The soil selected for inoculation by these samples was sandy, poor in humus and rich in lime with a gravelly subsoil.

The inoculations which were made have shown that the lupines and serradellas, which, up to that time, had never been developed successfully in the fields where the experiments were made, in spite of most careful culture, showed a remarkable growth in comparison with the plots which were not inoculated. The serradella which was inoculated directly with the nitragin did not respond to this inoculation, but peas inoculated with the nitragin showed a remarkably luxuriant development of the plants, with the formation of radical nodules, in comparison with the plants cultivated at the same time and not inoculated. It is necessary, however, that these experiments with inoculation be repeated for several years before definite conclusions in regard thereto can be drawn. (l'Engrais, Vol. 12, 1897, p. 351.)

Experience has shown that preparations of nitragin do not retain their vitality indefinitely and, in order to secure the best results, should be used as soon as possible after manufacture. It is evident also that the particular source from which each sample of nitragin is prepared must be designated, and the farmer wishing to use it as a fertilizer must purchase those varieties which are suited to the crop he wishes to grow; otherwise, he may make the mistake of applying nitragin derived from peas or beans to a clover field, or the contrary.

An excellent review of the methods of preparing nitragin and the processes of its application is given by J. Augustus Voelcker, in the Journal of the Royal Agricultural Society of England, Vol. 7, third series, part 2, pp. 253 *et seq.* The method of obtaining the pure cultures of the nitrifying bacteria is as follows:

A plate of prepared gelatin is inoculated from the nodules of the leguminous plants containing the living bacteria. A second plate is inoculated from one of the colonies formed in the gelatin plate. This process is continued until a pure cultivation is obtained, due to one particular species of nitrifying organism.

In preparing nitragin for commercial use the pure cultivation, obtained as above, is placed in a bottle, holding from 8 to 10 ounces, containing a small quantity of agar gelatin on which it is allowed to grow. The bottle is sealed and the contents kept in the dark. Up to the present time nitragin has been prepared from 19 different kinds of leguminous plants. Each bottle, when sealed, has a different colored label according to the kind of crop it is intended for, and also the German and botanical name of the plant. The contents of a single bottle are sufficient for securing the inoculation on an acre and a-half of land on which the crop is to be grown and are sold for about 65 cents. In appearance, as a rule, the bottle seems to be filled to the depth of one and a-half inches with a light brown jelly in which may be noticed a white growth or mold. Care must be taken that the temperature of the mixture be not raised above blood heat and that the bottle be not exposed for any length of time to strong light. A moderately high temperature and exposure for any length of time to intense light destroy the vitality of the organism.

The nitragin may be used directly on the the seeds which are to be sown, which, on germinating, develop radicles on which the organisms grow. In the inoculation of the seed the contents of the bottle of nitragin are liquefied by gentle warmth and poured into half a liter of lukewarm water. Any

residual jelly in the bottle is dissolved by shaking it with the water. When the jelly has been thoroughly distributed throughout the water the seeds to be inoculated are sprinkled thoroughly and worked well together so that a portion of the moisture is attached to each seed. The seeds are dried by mixing with some fine earth taken from the field in which they are to be sown.

The best method of applying nitragin is to at first introduce it into a sufficient quantity of moist earth, which is subsequently thoroughly stirred from time to time until the organisms have had time to multiply and distribute themselves in great numbers throughout the whole mass. This moist mass, dry enough, however, to permit of its being thoroughly stirred without caking, is applied to the field either by sowing broadcast or in ordinary drills such as are employed in the distribution of fertilizers. While, as has been said, the first effects have not been so good as have been anticipated, there is sufficient evidence to warrant the belief that the use of nitragin may in the near future become commercially valuable. This leads to the hope that we may find speedily verified the prediction which I made some four or five years ago to the effect that the nitrifying organisms of the soil, in the form of reasonably pure cultures, would eventually be used for fertilizing principles. The seeding of the soil with appropriate nitrifying ferments is certain to become as much of an exact science as the use of the proper ferments in butter and cheese making, in the curing and fermentation of tobacco and in other commercial operations where the activity of bacteria conditions the character and value of the product.

Mazé has recently shown that the life of bacteria resident in the nodules of the Leguminosæ is not a pure symbiosis. It has been demonstrated by this investigator that where artificial conditions, suited to the nourishment of these bacteria, are provided, they are able to oxidize free nitrogen in an environment from which all plant life is rigidly excluded. The bouillon in which the bacteria were cultivated was obtained from white beans. To this bouillon 2.5 per cent. of sugar and 1 per cent. of common salt and a trace of bicarbonate of soda were added. The bouillon was solidified by the addition of 15 per cent. of gelose and was spread in layers 4 millimeters thick on the botton of glass dishes about 20 centimeters in diameter. These vases were so disposed as to be supplied with a current of air from which every trace of oxidized nitrogen was removed and which had been subjected to a high temperature for a sufficient length of time to entirely sterilize it. This was accomplished by passing it through a tube containing metallic copper heated to low redness but not high enough to sensibly diminish the content of oxygen in the air. It was then conducted through a tube filled with broken glass saturated with sulfuric acid for the purpose of absorbing any ammonia, next through a bottle containing sterilized distilled water to saturate the air with the vapor of water, whence it passed to the dishes where the cultures were made. In five days it was found that the sugar of the broth was all consumed and that the quantity of oxidized nitrogen in the bouillon had been more than doubled.

Thus it was proved that the bacteria of the Leguminosæ, placed in a medium resembling as nearly as possible that in which they naturally live, are capable of oxidizing free nitrogen without any symbiotic help of any kind. If these deductions of Mazé be verified by subsequent investigators, it will prove that the nodules in which these bacteria reside are only convenient places in which they exercise their activity, which is entirely independent of the vital activity of the plant which they inhabit.

The sources of the first organic nitrogen

suitable to the nourishment of plants have been the subject of investigation in many quarters. It has been established with a considerable degree of certainty that the nitrifying organisms are capable of existing on the surface and even to a considerable depth in the interior of bare rocks at high altitudes where even the mosses and lichens fail to grow. It is evident, therefore, that these organisms have a great deal to do in the incipient stages of vegetable life and in the preparation of the first particles of humus, which is the substance distinguishing soil from finely cominuted rocks. Later investigations show also that nitrogen exists in combination with metals, as metallic nitrids, as has been shown by the investigations of Hillebrand and others. Among other metallic nitrids that of thorium has been detected. Notable quantities of mineral nitrogenous compounds have been found in the carnallit coming from the Stassfurt mines. As much as .018 per cent of ammoniacal nitrogen has been found in these salts. The artificial carnallit is richer in ammonia than that of nature. It is evident that in carnallit the ammonia replaces a small portion of the potash.

In regard to the origin of this ammoniacal nitrogen, it is generally understood that it comes from the decomposition of the living beings which peopled the sea whose evaporation produced the saline deposits. The ammoniacal nitrogen which is present in the primitive rocks, however, cannot be ascribed to this source, since these rocks were formed at an epoch when life did not exist upon the surface of the earth. This ammoniacal nitrogen, as has been said, occurs almost uniformly as metallic nitrids. It was doubtless, therefore, the first form of nitrogen used to nourish the beginnings of animal and vegetable life, since it existed before any of these forms could, by their decomposition, have furnished available nitrogen for plant growth. This ammoniacal nitrogen, therefore, must have served directly to nourish the first forms of life and thus to have helped lay the foundations of the whole vegetable world. (l'Engrais, Mar. 12, 1897; Apr. 9, 1897.)

A heated discussion has arisen between the French and German schools of agriculture in regard to the harmfulness of the denitrifying organisms found in soils and manures. Wagner urges the importance of sterilizing stable manure in order to prevent the loss of nitrogen that would otherwise be brought about by the organisms contained in it. Deherain, on the other hand, declares that this precaution is unnecessary when stable manure is applied to ordinary soils in the usual quantities.

Comparatively little attention has been given to the isolation and study of pure cultures of the nitrate-destroying organisms found in soils, manures, straw and fodders. While their existence has been repeatedly proved and their behavior in mixed cultures has been studied by Gayon and Dupetit, Springer, Deherain and Maquenne, Breal and others, the first denitrifying organisms obtained in pure cultures and accurately described were those reported by Burri and Stutzer in 1895. These investigators found a denitrifying organism in horse manure which they called *Bacillus denitrificans I.*, and which they found to rapidly destroy nitrates when growing in the same culture with *B. coli communis*. They also isolated and described, under the name of *B. denitrificans II.*, a denitrifying organism from straw.

A second denitrifying organism was found in horse manure by Schirokikh early in 1896, while more recently an organism of this class was found in cow manure by Ampola and Garino.

Considerable progress in our knowledge of the denitrifying ferments in soil has been made by Ewell in the Division of Chemistry, Department of Agriculture, in

investigations not yet published. He has separated and begun the study of three organisms that rapidly destroy nitrates with the formation of free nitrogen. One was obtained from a sample of soil, another one from pig manure and the third one from hen manure. The first two belong to the class of organisms which liquefy gelatine and produce a green or yellowish green fluorescent pigment. They belong to the same or closely related species. For comparison of these organisms, cultures of all similar organisms obtainable have been procured from the bacteriological laboratories of the United States Marine Hospital Service and of the Surgeon-General of the Army. The organisms thus far examined in regard to this property are the following: Two cultures from different sources of *B. pyocyaneus*, two of *B. fluorescens liquefaciens* and one each of *B. pyocyaneus*, *B. pyocyaneus D.* and *B. pyocyaneus pericarditidis*.

From the description given by Schirokikh of the organism found by him in horse manure it would appear that it is also of this class.

The study of the organisms isolated by Ewell will be continued and reported at the proper time; the investigation is to be extended to include soils of the various types, the feces of all the domestic animals, and various fodders, etc., in order that we may develop as fully as possible our knowledge of the nature and habitat of all organisms possessing the power to reduce nitrates with the liberation of free nitrogen.

Numerous bottles of nitragin have been received in this country, and I believe experiments are now in progress in many of our experiment stations in its use. The practical demonstrations which have been made, however, of its utility have been made at European stations, and many of the results which have been obtained in this country have not yet been published. Many interesting contributions to the literature of the subject will doubtless come from our own stations in the near future. Important work has already been done in studying the nodules of leguminous plants in many of our stations, especially in those of Massachusetts, Louisiana and Illinois.

(*To be concluded.*)

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THE AMERICAN SOCIETY OF NATURALISTS.

THE annual meeting of the 'Naturalists' and 'Affiliated Scientific Societies' was held at Cornell University, Ithaca, New York, December 28, 29, 30, 1897. The mild weather and attractive surroundings, together with the unbounded hospitality of the people of Ithaca and an excellent program and large attendance, combined to make the meeting more than usually successful.

In the absence of the President, Professor Whitman, of the University of Chicago, the chair was occupied by Professor S. F. Clarke, of Williams College, one of the founders of the Society. After listening to the Report of the Treasurer, action was taken on certain items of business.

Communications from the President of Columbia University, the President of the American Museum of Natural History, and the Secretary of the New York Academy of Sciences, inviting the Society to hold its next meeting in New York City, were read and referred to the Executive Committee. The Society subsequently decided to accept the invitations from New York.

It was reported that President McKinley was about to appoint a commissioner to serve in the place of Mr. John J. Brice and, in view of the present deplorable condition of the scientific work of the Commission, the following resolutions were unanimously adopted: